Chapter 5

CHEMISTRY AND DISTRIBUTION OF AMINO SUGARS IN SOILS AND SOIL ORGANISMS

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I. INTRODUCTION 198
II. CHEMISTRY OF AMINO SUGARS 198
III. DISTRIBUTION OF AMINO SUGARS IN LIVING ORGANISMS 200
  A. Invertebrates 200
  B. Fungal Cell Walls 201
  C. Bacterial Cell Walls 201
  D. Algal Cell Walls 204
  E. Higher Plants 204
  F. Antibiotics 204
IV. DETERMINATION 205
  A. Hydrolysis 205
  B. Estimation 206
  C. Separation 208
V. QUANTITY AND FORM OF AMINO SUGARS IN SOILS 208
VI. TURNOVER OF AMINO SUGARS IN SOILS 212
REFERENCES 215

I. INTRODUCTION

Amino sugars have been identified in the cell walls of bacteria and fungi, in bacterial extracellular polysaccharides and antibiotics, and in insect exoskeletons and other animal tissues. They have never been found in the cell wall structural components of higher plants although they are present in trace quantities in other plant components. Consequently their presence in soils, first reported by Bremner (1949), is of considerable interest because they provide evidence that much of the soil N is of microbial rather than plant origin. Since the end of the last century, chitin, a polymer of N-acetylglucosamine, has been recognized as an important component of fungal cell walls and insect exoskeletons. However, in the 1950s a combination of events, the development of paper chromatography and the search for new antibiotics, many of which contain amino sugars, led to the identification of a large variety of amino sugars and their derivatives and to the realization that their occurrence is far more widespread in living organisms than originally imagined. Improved analytical techniques have since greatly increased the rate of discovery.

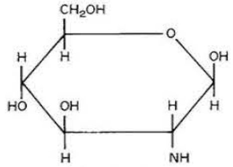
Organisms living in or on soils provide the starting materials for the humification process, and soil organic matter comprises a mixture of these with humus, the end product of a complex series of biochemical transformations. To anticipate the type of amino sugars we might expect to find in soils it will help to survey the naturally occurring compounds and the polymers in which they exist.

II. CHEMISTRY OF AMINO SUGARS

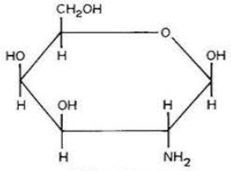
The 2-amino aldohexoses are the commonest, naturally occurring forms, but a 4-amino aldopentose (Volk, et al., 1970), a 2-aminohexuronic acid (Nasir-du-Din and Jeanloz, 1976) and a diamino sugar (Roppel et al., 1975) have been reported in bacterial cell walls. Sialic acid (N-acetyl-5-amino-3,5-dideoxy-D-glycero-D-galactononulosonic acid), a 9-carbon sugar, is widely distributed in animal tissues, but the evidence for its occurrence in other organisms is conflicting, possibly because of difficulties in identification. Aaronson and Leslie (1960) have identified it in cell walls of gram-negative bacteria while Warren (1963) has reported its sporadic distribution in bacteria and absence from plants and fungi. Ellwood (1970) found it in only 3 out of 63 gram-negative bacteria, but not in gram-positive walls. Occasional reports of its presence in higher plants have been made (Mayer et al., 1964; Schettino et al., 1970).

The naturally occurring 2-amino sugars are generally N-acetylated, but N-methyl, N,N-dimethyl, and N-nitroso derivatives are found in antibiotics (Brimacombe, 1971), while N- and O-sulfated sugars occur in animal tissues (Lindahl, 1976) and O-sulfated (Reistad, 1977), N-glycolyl (Azuma et al., 1970), and O-phosphorylated sugars (Nasir-du-Din et al., 1977) have been reported in bacterial cell walls. Sugars with the amino group on the C-3, C-5, and C-6 carbon atom occur in antibiotics (Brimacombe, 1971) and a C-4 amino sugar has been isolated from a bacterial cell wall (Dietzler and Strominger, 1973). Horton (1969) has discussed the chemistry and synthesis of amino sugars and their derivatives and has produced a table of their physical constants. Sharon (1965) has reviewed the distribution of amino sugars in living organisms.

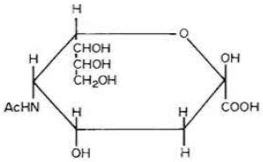
In quantitative terms, the three most important amino sugars are glucosamine (2-amino-2-deoxy-D-glucose), galactosamine (2-amino-2-deoxy-D-galactose), and muramic acid [2-amino-3-O-(D-1-carboxyethyl)-2-deoxy-D-glucose]. Their structures and that of sialic acid are given below.



β-D-glucosamine

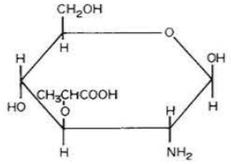


β-D-galactosamine



sialic acid

(N-acetyl-5-amino-3, 5-dideoxy-D-glycero-D-galactononulosonic acid)



muramic acid

(3-O-D-lactyl-β-D-glucosamine)

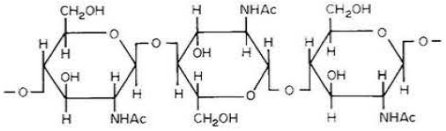
They are amphoteric primary amines which exhibit the normal reactions of monosaccharides; glucosamine has an isoelectric point between pH 8 and 10 which Verlinger (1926) calculated to be 9.9. In aqueous solution the acid salt is more stable than the free base which reacts with ammonia to form pyrazine derivatives (Taha, 1961) and is deaminated in sodium phosphate solution, pH 11.4, to yield ammonia (Tracey, 1952). The free amino group reacts with aromatic aldehydes to form Schiff bases which can be used for chemical identification.

Kent and Whitehouse (1955) suggest the amino group in the C-2 position, next to the reducing group, is analogous to the α-amino acids of the D-series. Protein amino acids have the L-configuration but those in the peptidoglycans in gram-positive cell walls are D-amino acids linked to an amino sugar backbone (Ikawa and Snell, 1956). This observation appears more than a coincidence, but in glycoproteins from other sources, where amino sugars provide the link between carbohydrate and protein, the latter is composed of L-amino acids.

III. DISTRIBUTION OF AMINO SUGARS IN LIVING ORGANISMS

A. Invertebrates

One of the earliest reported sources of glucosamine was chitin from the hard invertebrate exoskeleton. Chitin is a homopolymer of N-acetylglucosamine (N-acetyl-2-amino-2-deoxy-D-glucose) with a $\beta(1 \rightarrow 4)$ linkage in which the regular structure confers a high degree of crystallinity, as shown by X-ray diffraction studies. It is chemically very stable, being resistant to oxidizing agents such as H_2O_2 and ClO_2, and is insoluble in water and most other solvents. Chitin is insoluble in boiling 1.25M NaOH, but prolonged treatment may cause deacetylation. Cold concentrated HCl will dissolve it, but if the temperature is allowed to rise to 20°C, some reduction in chain length occurs (Rupley, 1964). Like cellulose, chitin is insoluble in cold anhydrous formic acid, but it dissolves on heating. Strout et al. (1976) have found chitin to be soluble in a mixture of formic acid and 2 M HCl at 12°C with no apparent change in chain length over a period of several hours.



Chitin

The remarkable chemical stability and physical strength of chitin make it an ideal structural component. In the inner, soft layer of the insect cuticle chitin is covered with tanned protein. Crustacean shells are formed by the deposition of calcium carbonate in a matrix of chitin microfibrils and protein. Chitin is invariably associated with protein, if not linked to it (Rudall, 1976). Attwood and Zola (1967) have concluded that the persistence of amino acids after prolonged alkali treatment provides strong evidence for covalent bonding between chitin and protein.

The distribution of chitin in the animal kingdom has been reviewed by Richards (1951). It occurs predominantly in the body walls of the Arthropoda which, in terms of numbers, is the largest phylum of the animal kingdom and has many representative genera in soils. Sharon (1965) has collected data which show that chitin accounts for about one-third, but rarely more than one-half, by weight of the insect exoskeleton, the other major component being protein. Chitin is also found in the gut lining of worms and as an important constituent of nematode eggshells (Bird and McClure, 1976).

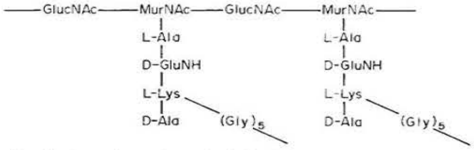
B. Fungal Cell Walls

The cell walls of fungal mycelia and yeasts are complex, multilayer structures composed of 80 to 90% polysaccharide. The two dominant polysaccharides are chitin and cellulose, but they rarely occur together and the presence of one or the other has formed the basis of an early taxonomic division. Later work has shown they are laid down as microfibrils in an amorphous polysaccharide matrix. On the basis of cell wall composition, Bartnicki-Garcia (1968) has divided the fungi into eight categories. The presence of cellulose is characteristic of most of the lower fungi, but by far the largest category, which includes the higher fungi, is based on a wall composition of chitin and glucan. Chitosan, a nonacetylated polymer of glucosamine, has been identified as the dominant component along with chitin in the walls of Mucor rouxii (Bartnicki-Garcia and Nickerson, 1962). A partially N-acetylated polymer of galactosamine and galactose has been reported to occur in varying ratios with chitin in the mycelial walls of several fungi (Distler and Roseman, 1960; Bandalaye and Nordin, 1976).

Fungal chitin appears to be similar in all respects to α-chitin, one of three forms identified by X-ray diffraction in invertebrates. Chemical analyses have indicated that in fungal cell walls it is closely associated with other polysaccharides. Electron microscope studies by Hunsley and Burnett (1970) have suggested a layered structure with protein permeating the chitin layer.

C. Bacterial Cell Walls

Bacterial cell walls are structurally more complex than their fungal counterparts and they contain a greater variety of amino sugars and their derivatives. Their structure and biosynthesis have been reviewed by Osborn (1968). The composition of gram-positive walls differs from that of gram-negative, but the one group of polymers common to both is the peptidoglycans, which give the walls their rigidity. The peptidoglycans consist of a backbone of alternating N-acetylglucosamine and N-acetylmuramic acid residues cross linked by peptide chains, attached through the N-terminal group to the carboxyl of the O-lactyl ether. This group of polymers has been given various names-murein, mucopeptide, and glycopeptide- but peptidoglycan is perhaps the most logical and generally accepted name.



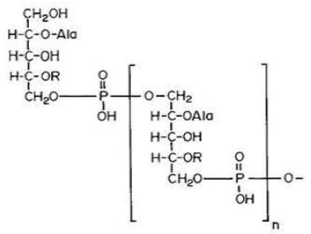
Peptidoglycan from the wall of Staphylococcus aureus

GlucNAc = N-acetylglucosamine
MurNAc = N-acetylmuramic acid

The glycan structure of N-acetylglucosamine linked to N-acetylmuramic acid by a $\beta(1 \rightarrow 4)$ linkage is remarkably uniform in all bacterial cell walls and differs from chitin only by the presence of the O-lactyl ether. The variety of peptidoglycan structures appears to arise from the composition of the peptide chains and has been discussed as a basis for taxonomic classification by Schleifer and Kandler (1972). Peptidoglycans account for over half the dry weight of most gram-positive walls, the remainder being protein - teichoic acids or acidic polysaccharides or both.

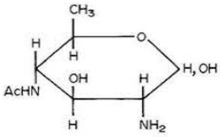
Teichoic acids are also major components, accounting for 20 to 50% of the dry weight but they are rarely if ever found in gram-negative walls. Baddiley (1972) in a review used the term to include all bacterial wall, membrane, and capsular polymers containing glycerol phosphate or ribitol phosphate residues. Teichoic acids are water-soluble polymers linked to other components; in the cell wall they are covalently linked to peptidoglycans. Ribitol teichoic acids, found only in cell walls, are polymers of ribose units linked by phosphate diester bridges between C-1 and C-5, with alanine and N-acetylglucosamine as substituent groups attached to ribose. Glycerol teichoic acids are more variable and widespread in occurrence. They are polymers of glycerol linked by phosphate diester bridges in which glycerol may be partially replaced by N-acetylglucosamine. Other glycerol teichoic acids have been isolated in which each alternate glycerol has been replaced by N-acetylglucosamine and another related polymer composed solely of N-acetylglucosamine units linked by a phosphate diester between carbons 1 and 4 (Archibald et al., 1968).

Information on the composition of wall polysaccharides is limited, but many are known to contain amino sugars. For example, polysaccharides



Ribitol teichoic acid from the wall of
Staphylococcus aureus
R = α or β-N-acetylglucosamine
Ala = D-alanine

isolated from streptococcal walls contain either N-acetylglucosamine or N-acetylgalactosamine (Pazur et al., 1971), and a diamino sugar, 4-N-acetamido-2-amino-2,4,6-trideoxy-D-glucose, has been identified in wall polysaccharide of *Bacillus licheniformis* (Zehavi and Sharon, 1973). The structure of gram-negative walls is not so clearly understood. Peptidoglycans are less important, accounting for 10% or less of the dry weight. Lipoprotein is present and lipopolysaccharide appears to be confined entirely to gram-negative walls.



4-N-acetamido-2-amino-2, 4, 6-trideoxy-D-glucose

Lipopolysaccharide is composed of a core, which frequently contains glucosamine, to which the lipid and an O-specific side chain of polysaccharide are attached. The latter contains the determinants of the O-antigen and shows a wide diversity in composition. Various amino sugars have been isolated from O-specific side chains including glucosamine, galactosamine, and mannosamine (Osborn, 1968), arabinosamine (Volk, et al., 1970), fucosamine (Crumpton and Davies, 1958), and a diamino sugar (Wilkinson, 1977). Capsular polysaccharides are produced by a number of bacteria and play an important role in immunological reactions. Many of these contain amino sugars, primarily glucosamine and galactosamine.

D. Algal Cell Walls

Algal cell walls were at one time thought to contain chitin, but this was never substantiated. Cellulose and various polyuronides, some of commercial importance, are the dominant polysaccharides, but the walls of the blue-green algae show a remarkable similarity to those of bacteria (Echlin and Morris, 1965) through the presence of peptidoglycans. Peptidoglycans are confined to the basal layer of the wall, similar to gram-negative walls, but the amount is much greater, being of the order of 50% of the dry weight (Drews, 1973). They show a further similarity to gram-negative walls in the presence of lipopolysaccharide. Weise et al. (1970) have identified a 7-carbon amino sugar, 2-amino-2-deoxy heptose, in lipopolysaccharide isolated from Anacystis nidulans.

E. Higher Plants

The dominant role of glucosamine in living organisms appears to be in structural components, and yet it is absent from cell walls of higher plants where glucose fulfills the equivalent function and has many common properties. However, amino sugars have been identified in higher plants fulfilling their other important function, that of providing a link between carbohydrate and protein components of glycoproteins (Sharon, 1974). They are mainly confined to seeds; glucosamine and galactosamine have been found in wheat endosperm (Mares and Stone, 1973), in corn pericarp (Boundy et al., 1967), in rapeseed (Goding et al. 1970), and in legume seeds (Ericson and Chrispeels, 1973). They have also been reported in water extracts of the leaves of Cannabis sativa (Hillstead et al., 1977). Plant lectins, whose functions are not fully understood, are glycoproteins, many of which contain glucosamine (Goldstein and Hayes, 1978). A number of enzymes found throughout the plant and animal kingdoms are now known to be glycoproteins (Pazur and Aronson, 1972) and contain amino sugars.

F. Antibiotics

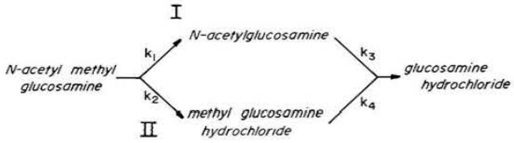
Antibiotics, produced by bacteria, actinomycetes, and fungi, show a great diversity of structure. Bérdy (1974) has proposed a system of classification based on structure. The largest group contains those composed of carbohydrate, three classes of which are based on the presence of monoamino sugars, aminodisaccharides, or aminoglycosides. It is impossible to attempt a survey here of the great number of amino sugars in antibiotics. These have been adequately covered by Bérdy (1974), Umezawa (1974), and Umezawa (1976).

IV. DETERMINATION

A. Hydrolysis

Amino sugars occur in polymers in soils; there has been only one report in the literature of the identification of the free sugar (Skujiņš and Puķite, 1970). Estimation, generally based on a colorimetric method, is made on the individual amino sugar following cleavage of the glycosidic bonds. Alkaline hydrolysis has little effect on these bonds, so mineral acids are used and their success depends on a balance between hydrolysis and decomposition of the products. The stability of amino sugars in acid solution is variable; sialic acid and arabinosamine decompose in weak acids, but muramic acid is very stable, even at elevated temperatures. Glucosamine is slightly more stable than galactosamine.

Moggridge and Neuberger (1938) examined the hydrolysis of methylglucosamine and N-acetylmethylglucosamine in HCl at 100°C and have suggested two possible pathways:


I
N-acetylglucosamine
k1
k3
N-acetyl methyl glucosamine
glucosamine hydrochloride
k2
k4
methyl glucosamine hydrochloride
II

The rate constants k_1, k_2, and k_3 are approximately equal but about 250 times greater than k_4, due to electrostatic shielding of the glycosidic bond on C-1 by the $-NH_3^+$ on C-2. Choice between pathways I and II depends upon configuration of the glycoside and the type of substituent on the amino group (Foster et al. 1956). Pathway I is the faster but inevitably some deacetylation occurs and hydrolysis slows down. Hellerqvist and Lindberg (1971) have used 80% acetic acid as the hydrolytic agent to protect the N-acetyl group in bacterial lipopolysaccharide, but subsequent O-deacetylation caused decomposition. Trifluoroacetic acid has been used to hydrolyze plant glycoprotein (Yasuda et al., 1970). Johansen et al., (1960) argue on theoretical grounds that hydrolysis of glycoside bonds is favored by high temperature and high acid concentration but the latter causes partial deamination. Glucosamine is stable in 2 M HCl at 100°C for 15 hr, but decomposition increases with increasing acid strength above 2 M (Boas, 1953). Decomposition can be minimized by use of a wide acid to substrate ratio and by performing the hydrolysis under N_2 (Walburg and Ward, 1963). Anastassiadis and Common (1958) have maintained a high H^+ ion concentration without the disadvantages of a strong mineral acid by using a cation exchange resin in 0.05 M HCl, but Kim et al. (1967) reported 10% destruction under these conditions.

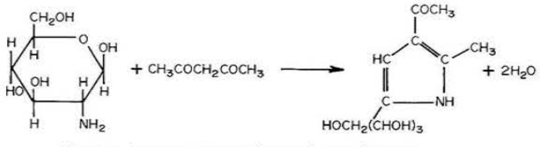
Numerous hydrolytic procedures, based on permutations of the three variables - time, temperature, and acid strength - have been reported in the literature, but there can be no universal method. Each represents a compromise between cleavage of the glycosidic bond and acid destruction of the products. Hydrochloric acid is the most favored acid, and two examples are given here. Smithies (1952) examined the composition of fungal walls as a means of predicting components of soil organic matter, and obtained maximum release of amino sugar with 6 M HCl at 100°C in 6 hr; approximately 20% was destroyed. Optimum hydrolysis of bacterial cell walls, also an important source of soil N, has been achieved with 6 M HCl at 100°C in 2 hr (Salton and Pavlik, 1960), and extension beyond this period has led to low recovery of amino sugar.

A colorimetric method, independent of breaking the glycoside bond, can be used (Tsuji et al., 1969). The method, based on deamination, only requires the amino group to be free so that success depends on complete removal of the N-substituent. This may not be the sole criterion, as values for chitosan have been obtained which are lower than those using the same method following hydrolysis (Tsuji et al., 1970). Ride and Drysdale (1972) have adapted this procedure to determine chitin, following deacetylation in alkali, as a measure of fungal biomass in plant material, but the method has since been criticized by Sharma et al. (1977).

Most of the studies on amino sugars in soils have been based on the assumption that chitin is the dominant source and, as a result, Smithies' (1952) method, using 6 M HCl at 100°C, has formed the basic pattern for hydrolysis. Stevenson (1957a) has reported the optimum period to lie between 6 and 12 hr for surface soils and has recommended 9 hr as a suitable compromise. Sowden (1959) has estimated total amino sugar, glucosamine and galactosamine liberated over 8 to 40 hr and has obtained a linear relationship between log of concentration and time. Extrapolation to zero time has provided correction factors for an 8 hr hydrolysis, of 1.21, 1.25, and 1.26 for total amino sugar, glucosamine, and galactosamine, respectively. These values are very similar to those used by Bremner and Shaw (1954) and Stevenson (1957a). In all three studies a ratio of acid to soil of 10 ml to 1 g was used. In a variation on the method, Stevenson (1957a) has been able to reduce the time of maximum release of amino sugar from 18 to 4 hr for a B-horizon sample of a planosol by autoclaving at 1 bar and 120°C, but decomposition increased to 34%. Parsons and Tinsley (1961) have reduced hydrolysis time to 1 hr by using a 1:1 mixture of 6 M HCl and 90% formic acid.

B. Estimation

The most commonly used colorimetric method for the estimation of amino sugars is that of Elson and Morgan (1933), which requires both the amino group and the OH on C-1 to be free. The assay is based on the reaction with alkaline acetylacetone (pentane-2,4-dione) to form pyrrole derivatives (Cornforth and Firth, 1958) which develop a stable red color with 4-dimethylaminobenzaldehyde. The chemistry of the method is complex (Horton, 1969), and this has led to the development of numerous published modifications. Belcher et al. (1954) have critically examined the variables which influence color formation and stability.



Reaction between glucosamine and acetylacetone

Crumpton (1959) has measured relative color intensities produced by a range of amino sugars; values for glucosamine and galactosamine are similar, but those for mannosamine and muramic acid are much lower. N-acetylglucosamine also produces less color and, like muramic acid, has different absorption maxima; a separate method has been developed for the former compound (Morgan and Elson, 1934). Neutral sugars and amino acids cause no interference on their own, but mixtures of the two produce a color when heated with 4-dimethylaminobenzaldehyde in alkaline solution (Sideris et al., 1938). The degree of interference depends on the method used; Belcher et al. (1954) have reported that a 20-fold excess of a sugar-amino acid mixture caused no interference in their procedure. As a precaution, neutral sugars can be removed by passing the solution through a cation-exchange resin and eluting the amino sugars with 2 M HCl (Boas, 1953). A more serious problem with soil hydrolysates is the formation of a colored complex between iron and acetylacetone. This can be prevented either by precipitation of iron on neutralizing the hydrolysate with an anion exchange resin, which also absorbs humin (Stevenson, 1957a), or by solvent extraction with acetylacetone-chloroform (Sowden, 1969) or methyl isobutyl ketone (4-methylpentan-2-one) (Beckwith and Parson, 1980).

Colorimetric methods using ninhydrin (Moore and Stein, 1948) or 2,4,6-trinitrobenzene-1-sulfonic acid (Galambos and Shapira, 1966) are only of value in the absence of amino acids. Dische and Borenfreund (1950) have developed a colorimetric method based on deamination, but neutral sugars also produce a color. Tsuji et al. (1969), as mentioned earlier, have also used the deamination step but produced a color with 3-methyl-2-benzothiozolane hydrazone hydrochloride and ferric chloride. The method is claimed to be free from interference from neutral sugars and amino acids. However, for soil hydrolysates, iron may well interfere in the deamination reaction and should be removed.

Bremner and Shaw (1954) have improved the alkaline distillation method of Tracey (1952), and it has since become one of a series of distillation techniques used for analysis of soil hydrolysates (Bremner, 1965).

C. Separation

Individual amino sugars can be separated quantitatively most effectively by ion exchange chromatography (Crumpton, 1959), and analyzers can be programmed to separate them from amino acids as well. Some care must be exercised in identifying peaks, since iron and aluminum can alter retention times (Sowden, 1969). Amino sugars in the eluate are estimated colorimetrically, but an interesting separation using ligand exchange chromatography relies on direct detection by measuring absorbance at 254 nm of amino sugar-copper chelates (Navratil et al., 1975). Development of physically stronger resin beads will speed up separation by allowing the use of increased pressure.

Gas-liquid chromatography (GLC) allows faster separation and direct estimation, but the production of volatile derivatives takes time. Trimethylsilyl (TMS) derivatives, based on the early work of Sweeley et al. (1963) and Perry (1964), are the most widely used. The derivatives are easily prepared following an initial N-acetylation step, and amino sugars and neutral sugars can be separated without the interference of amino acids. Casagrande and Park (1977) have used TMS derivatives for the separation of muramic acid. Other volatile derivatives used are acetates (Jones et al., 1962), N-carboxyethyl and O-TMS derivatives (Oates and Schrager, 1967) and, in one of the few soil (peat) studies, trifluoroacetyl derivatives (Casagrande, 1970).

The sensitivity and efficiency of GLC is greatly superior to ion exchange, so much so that α- and β-anomers are separated as two and sometimes three peaks for each component. This can be overcome by reduction of the reducing group to an aminoglycitol prior to acetylation (Perry and Webb, 1968). Metals present in soil hydrolysates interfere in the production of volatile derivatives, and methods have been developed to remove them prior to derivative formation (Cheng, 1975; Thom, 1976).

V. QUANTITY AND FORM OF AMINO SUGARS IN SOILS

Estimates of amino sugar N in surface horizons of soils from a range of climatic regions vary between 1.4 and 11.7% of the total N (Table 1). The widely quoted values of 5 to 10% are based on the results of Bremner and Shaw (1954), Stevenson (1957b), and Sowden (1959). Most of the values have been obtained after a 6-hr hydrolysis, using the method of Smithies (1952), and applying a correction factor. Sowden has used a longer hydrolysis period and has extrapolated to zero time. There are few obvious

TABLE 1 Amino Sugar N Content of Soils Expressed as a Percentage of Total N

Geographical Location	No. of Soils	Amino Sugar N (% of Total N)	Reference
United Kingdom	6	4.9-10.2	Bremner and Shaw (1954)
United States	19	6.0-11.7	Stevenson (1957b)
Canada	8	4.4-10.0	Sowden (1959)
India	5	2.4-5.6	Singh and Singh (1960)
Germany, Austria, and Belgium	17	3.9-7.4	Cheng and Van Hove (1964)
Sweden	1	10.8	Nõmmik (1967)
Canada	3	3.6-5.2	Khan and Sowden (1971)
Germany	3	5.5-7.2	Aldag and Kickuth (1973)
Canada	7	1.4-7.0	Lowe (1973)
Dominica, St. Vincent	10	4.0-8.0	Sowden et al. (1976)
Israel and Sardinia	6	3.9-9.3	Chen et al. (1977)
Canada	118	5.3-7.0	Sowden (1977)
Tanzania	4	5.2-11.5	Singh et al. (1978)

relationships with other soil factors; values generally do not correlate with soil N content. Stevenson (1957b) has concluded, for a sequence of soils developed from loess, that the proportion of soil N in the form of amino sugar N increases as the degree of weathering increases. Lowe (1973) has found that the value increases markedly with humification in forest soils. Values for tropical soils quoted by Singh and Singh (1960) are low, but they may not have been corrected for loss during hydrolysis; values obtained by Sowden et al. (1976) are little different from those of other soils. Values for nonvolcanic tropical soils range from 1.0 to 10.9 and are very much higher than for volcanic soils, 0.8 to 3.0 (Dalal, 1978). Salomon (1963) failed to find any relationship between amino sugar N and aggregate size in cultivated soils.

The one obvious trend, reported by Stevenson (1957b) and confirmed by Sowden (1959) and Nõmmik (1967) is the increase in percentage amino sugar N of total N down the profile for a number of soils, values reaching in excess of 20% in the B-horizon. This accumulation is difficult to explain, but it may be associated with increased clay content. Stevenson and Braids (1968) have suggested that, because the polymers which accumulate contain glucosamine rather than galactosamine, they may have originated from microbial activity in earlier geological periods (Stevenson and Tilo, 1968). A similar concentration trend has been reported for peat soil profiles (Sowden et al., 1978), but here galactosamine rather than glucosamine values increase.

The amino sugar N values quoted are total values and comprise the sum of glucosamine N and galactosamine N plus any other amino sugar N present. Glucosamine and galactosamine are the only amino sugars whose crystal structures have been positively identified following isolation from soil (Bremner, 1958). The polymer form in which amino sugars occur in soils was originally considered to be chitin. Waksman (1936) has quoted two reports from the late nineteenth century identifying substantial quantities of chitin in forest soils, but assessments were based on estimation of skeletal remains of insects (Bremner and Shaw, 1954). Although N-acetylglucosamine has been identified in trace quantities in soil hydrolysates (Stevenson, 1956; Lynch et al., 1957; Young and Mortensen, 1958), it is known to occur in polymers other than chitin and deacetylation is almost complete during hydrolysis. Chitin is soluble in hot formic acid and insoluble in alkali, but extraction studies have provided no clear evidence for its presence or absence. The percentage amino sugar N of the residue N, following hot formic acid extraction, was little different from that of the original soil (Parsons and Tinsley, 1961). Similarly, Bremner (1955a) found amino sugar N contents of humic acids, extracted from soils with 0.5 M NaOH, to be approximately the same as those of the original soils. On the other hand, values reported for humic acids extracted from three Alberta soils by Khan and Sowden (1971) were considerably lower than the soil values. Results for two inceptisols from Dominica, obtained by Sowden et al. (1976), have shown the proportion of soil N as amino sugar N to be marginally higher in the residue after alkali extraction than in the original soil and the glucosamine/galactosamine ratio to be the highest in the residue (see Table 2).

Chitosan and partially deacetylated chitin are acid-soluble and may pass into the fulvic acid fraction. Amino sugar N content of fulvic acids tends to be low, but Sowden and Schnitzer (1967) reported a glucosamine/galactosamine ratio of 3.1:1 for a fulvic acid compared to 1.6:1 for the corresponding humic acid from a podzol B_h horizon. Stevenson (1960) found that the fulvic acid fraction of a pyrophosphate extract (pH 9) contained more amino sugar N than that from a sodium hydroxide extract.

The best, but inconclusive, evidence for chitin in soils comes from glucosamine/galactosamine ratios. Sowden (1959) has found they range from 1.6:1 to 4.6:1, with the highest values obtained for acid soils under coniferous litter, where fungi predominate. Ratios calculated from the results of Lowe (1973) support this finding. For other soils the ratio is closer to unity, emphasizing the higher proportion of galactosamine which is generally associated with bacterial cell walls and extracellular polysaccharides (galactosamine is present in walls of a few fungal species).

TABLE 2 Amino Sugar Content and Glucosamine/Galactosamine Ratios for Two Soils and Fractions Isolated from Them

Soil No.	Soil	Humic Acid	Fulvic Acid	Insoluble Residue
	Amino Sugar N as % of Total N			
2	7.7	4.8	2.8	9.0
5	7.1	3.0	1.7	8.1
	Glucosamine/Galactosamine = Ratio			
2	1.6	1.3	1.2	3.5
5	1.4	1.3	1.4	2.5

Source: Sowden et al. (1976).

These results indicate that much of the amino sugar in soil is of bacterial origin. However, if this is so, why has muramic acid, present in peptidoglycans in amounts equal to glucosamine, been so rarely found? The only reports of its presence in soils are by Millar and Casida (1970) and Casagrande and Park (1978). The former identified it in all but 1 of 24 Californian soils examined. Sowden (1959) was unable to positively identify muramic acid in soil hydrolysates, thus raising the question of whether it survives the hydrolysis and cleanup procedures. Muramic acid possessing a carboxyl group, may be carried down with the precipitate of iron and aluminum hydroxides or may be held on an anion exchange resin. Millar and Casida (1970) failed to recover muramic acid quantitatively using the anion exchange method of Stevenson (1957a). The only other amino sugar of bacterial origin reported in soil is talosamine (Wang and Cheng, 1964).

Fractionation of soil extracts has failed to provide clarification of the nature of amino sugar containing polymers. Using cetavalon (cetyltrimethyl ammonium bromide), Parsons and Tinsley (1961) obtained a polysaccharide-rich fraction which contained most of the extracted amino sugar. Stevenson (1960), using a charcoal column, separated from fulvic acid a fraction in which 25.4% of the N was amino sugar N and this he considered to be a nitrogenous polysaccharide. A polysaccharide, isolated by Swincer et al. (1968a), contained almost equal quantities of glucosamine and galactosamine along with the four amino acids associated with peptidoglycans: alanine, aspartic acid, glutamic acid, and glycine. The content of amino sugar relative to neutral sugar increased with increasing molecular weight (Swincer et al., 1968b). Finch et al. (1968) extracted a polysaccharide containing approximately 2% amino sugar and a similar spectrum of amino acids. The general conclusion drawn from fractionation studies is that amino sugars invariably occur along with neutral sugars and amino acids and possibly other organic components.

Apart from muramic acid the only other evidence for the existence of peptidoglycans in soils is the presence of D-amino acids. Bremner (1950) estimated that 1.4 to 1.7% of total soil amino acids occur in the D-form. However, with the development of the more sensitive GLC methods, significantly larger quantities (approximately 5 to 16%) have been identified (Kawaguchi et al., 1976; Pollock et al., 1977).

VI. TURNOVER OF AMINO SUGARS IN SOILS

The quantities of muramic acid, glucosamine, and galactosamine in soils are far in excess of the amounts which would be expected in microbial cells (see Chap. 10). Thus we have to invoke a mechanism whereby amino sugars accumulate in soils, knowing that glucosamine and chitin are rapidly decomposed when added to soils (Bremner and Shaw, 1957; Okofor, 1966a).

If we assume the production of antibiotics in soils to be low, then the major sources of amino sugars are fungal and bacterial cell walls, extracellular polysaccharides, and insect integuments. Little is known of the enzymic hydrolysis of extracellular polysaccharides, but lysis of microbial cell walls has been extensively studied. Chitinase consists of an endochitinase, which splits off dimers, and chitobiase, which hydrolyzes the disaccharide to N-acetylglucosamine. Chitinase is found in earthworms (Tracey, 1951), nematodes (Tracey, 1958), termites (Tracey and Youatt, 1958), beans (Bahl and Agrawal, 1968), root and stem tissues of trees (Wargo, 1975), and in many soil microorganisms, notably the actinomycetes (Gray and Baxby, 1968). Chitin in fungal walls appears to be protected by a layer of glucan which must be removed to allow access to chitinase (Skujiņš, Potgieter, and Alexander, 1965). Consequently, most microorganisms capable of lyzing fungal walls produce a mixture of chitinase and $\beta(1 \rightarrow 3)$ glucanase. Despite the presence of a large number of lytic organisms in soils, a number of fungal genera survive in the hyphal form or as sclerotia or chlamydospores. Ballesta et al. (1969) have suggested that the resistance of Torulopsis spp. is due to an extra layer of a xylose-containing heteropolysaccharide which protects chitin and glucan from lytic enzymes.

Some fungi which survive adverse conditions are capable of melanin production (Lockwood, 1960), and Bloomfield and Alexander (1967) have presented evidence relating resistance to lysis with the presence of a layer of melanin. Melanin is distributed throughout the wall of Aspergillus nidulans and appears to form a complex with chitin (Bull, 1970a). In the sporophores of Daldina concentrica, the pigment forms crosslinkages with the amino groups of wall chitin (Allport and Bu'Lock, 1958). The walls of a melanin-forming wild type of Aspergillus nidulans are resistant to a mixture of chitinase and $\beta(1 \rightarrow 3)$ glucanase, but the hyaline filaments of a mutant are easily solubilized (Kuo and Alexander, 1967). The role of melanin in the stabilization of fungal walls may be as an inhibitor of the lytic enzymes or as a physical barrier to the enzymes. The activity of chitinase is certainly affected by the presence of melanin (Bull, 1970b); inhibition is time-dependent and of the noncompetitive type, but melanin does form a physical barrier which prevents reaction of wall polysaccharides with an optical brightener, Calcofluor (Gull et al., 1972). The resistance of some algal walls to microbial decomposition has been explained by the presence of a polyaromatic component similar to lignin which increases in concentration with age (Gunnison and Alexander, 1975).

Bacterial cell walls are disrupted by lysozyme, which attacks the β-glycosidic link of wall peptidoglycans producing peptides with N-acetylglucosamine and N-acetylmuramic acid still attached at the ends. Lysozyme also hydrolyzes chitin. Activity of the enzyme is dependent on the amino groups being acetylated and the hydroxyl groups being free. Many bacterial cells, produced during the stationary phase, are almost completely resistant to lysis, which may be due to loss of the N-acetyl substituent, as in Bacillus spp. (Hayashi et al., 1973) or to the presence of O-acetyl groups as in Lactobacillus spp. (Logardt and Neujahr, 1975).

Wagner and Mutatkar (1968) have provided evidence for the stability of microbial cell walls in soils. After 6 months incubation of ^{14}C-glucose with soil, much of the specific activity is concentrated in amino acids associated with peptidoglycans and in amino sugars, particularly glucosamine. Marumoto et al. (1974) found the major portion of intracellular amino acids is rapidly mineralized when dead Bacillus subtilis cells are incubated in sand, but the amino sugar fraction remains intact. ^{14}C-labeled pigmented fungal wall material is much more stable to microbial decomposition in soils than either hyaline walls or cytoplasmic contents (Hurst and Wagner, 1969). The stability of ^{14}C-labeled microbial material has also been reported by Shields et al. (1973), and of microbial walls, through indirect evidence, by McGill et al. (1975).

The stability of amino acids in soils has been explained by the formation of heterogeneous polymers through reactions with oxidized polyphenols (Flaig, 1950; Swaby and Ladd, 1962). Polyphenols produced by fungi may oxidize and polymerize, either with themselves or other metabolic products, to form "humic" polymers (Martin and Haider, 1971), and polymer production can be influenced by the presence of clays (Filip et al., 1972). Polymers produced in the laboratory from polyphenols and glucosamine and chitosan are much more resistant to decomposition than either glucosamine or chitosan by themselves (Bondietti et al., 1972). The chitosan-phenol polymer is alkali-soluble and acid-insoluble. Chemically pure chitin is easily degraded in soils (Bremner and Shaw, 1957; Okofor, 1966a), but chitin in insect wings is significantly more stable than deproteinized or dewaxed wings (Okofor, 1966b). Fragments of wing are still identifiable after 300 days, but deproteinized wing disappears rapidly.

Physical barriers in the soil may impede movement of microorganisms toward their food source. Thin layers of glass beads prevent decomposition of chitin by microorganisms (Ou and Alexander, 1974) and ^{14}C-labeled starch in soil pores, inaccessible to microorganisms, is comparatively resistant to decomposition (Adu and Oades, 1978). Stabilization of newly formed microbial cell components may be afforded either by adsorption on clay surfaces (Sørensen, 1972, 1975) or by adsorption of enzymes responsible for their degradation (Skujiņš et al., 1974).

Amino acids and amino sugars are rapidly synthesized during immobilization of labeled inorganic N (McGill et al., 1973) and they appear to accumulate in the same proportions, regardless of the type of organic material added (Sowden, 1968). Evidence from incubation experiments suggests the level of amino sugar N, as a proportion of total N, either remains constant (Bremner, 1955b) or increases with time (Sowden and Ivarson, 1959, 1974). In the case of deciduous litter compost, galactosamine increased relative to glucosamine over a period of 165 days, possibly reflecting the build up of a bacterial flora, although no muramic acid was identified (Sowden and Ivarson, 1959).

The biomass, which consists of microbial cells, is an important constituent of the labile nitrogen pool in soils (Jenkinson, 1966), but the comparative stability of microbial cell walls makes it difficult to estimate the contribution of amino sugars to the mineralization process. Cultivation causes a small loss of amino sugar N, but on average increases slightly the proportion of total N as amino sugar N (Keeney and Bremner, 1964). Halstead and Sowden (1968) obtained no relationship between nitrate production and amino sugar N content whereas Singh et al. (1978), from incubation studies, reported amino sugars to be the most labile fraction.

The evidence in the literature suggests amino sugars originate from microbial cell and insect structural components and that, based on the galactosamine content, there is more present than can be accounted for by living microbial cells. We know little about the amino sugar-containing polymers in soils, but the information available suggests they are closely associated with neutral sugars and amino acids. There is no evidence either for the presence or absence of chitin, but intuitively it is difficult to believe it is not present. Muramic acid is of interest, but the amount in mineral soils is lower than might be expected, although surprisingly there is more in swamp soils (Casagrande and Park, 1978). Considering the variety of amino sugars identified in living organisms, it is disappointing that only glucosamine and galactosamine have been positively identified in soils, but more careful work on isolation and improved analytical techniques may change this. Amino sugars are of significance in soils because, along with amino acids and nucleic acids, they are important components of the labile N pool. Millar and Casida (1970) have suggested muramic acid content might be used as a measure of the bacterial population (see Chap 10). Both qualitative and quantitative data indicate that the pattern of amino sugars is similar in soils formed under a wide range of conditions and, like that of amino acids, the apparent resistance of amino sugars to microbial decomposition is yet to be explained. Until we know more about the chemical and physical forms in which these compounds exist in soils, we cannot fully understand the biological processes of mineralization and immobilization in soils.

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